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Vaccines for bacterial sexually transmitted infections: A realistic goal?

(gonorrhea/chlamydia/syphilis/chancroid)

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ABSTRACT **Bacterial infections of the genital tract (gonorrhea, chlamydia, chancroid, syphilis) are common and cause significant morbidity. Their importance is heightened by recent appreciation of their roles in facilitation of transmission of the human immunodeficiency virus (HIV). Each is capable of causing repeated infections, suggesting lack of permanent broadly effective immunity. An effective vaccine has yet to be developed for any of these diseases. Rapid progress in understanding the molecular basis for pathogenesis of infection, including mechanisms for escape from otherwise effective immune surveillance and mechanisms for causing injury to host cells, has stimulated renewed efforts to make vaccines for some of these infections. Progress has been greatest for *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. Present emphasis is on the major or principal outer membrane proteins of *N. gonorrhoeae* and *C. trachomatis*, based on evidence for neutralizing antibodies directed against surface-exposed variable domains of each of these proteins. Other surface-exposed proteins, including the iron-repressible transferrin receptor in gonococci and certain heat-shock proteins in chlamydia, also may be targets for vaccines. Although much remains to be learned, cautious optimism is warranted.**

The majority of the world research effort on sexually transmitted infections is now focused on human immunodeficiency virus (HIV) infection, for the understandable reasons that HIV infections are spreading dramatically around the globe, are increasingly spread by heterosexual behaviors, and are ultimately lethal. Current treatment for HIV is only of modest benefit, and intense efforts to create an HIV vaccine have been thwarted to date by the antigenic variability of the virus.

The curable sexually transmitted diseases (STDs) caused by bacterial pathogens (*Neisseria gonorrhoeae, Treponema pallidum, Haemophilus ducreyi*, and *Chlamydia trachomatis*) have received relatively less research and clinical attention compared with HIV. Use of a combination of approaches, including public education and accessible treatment clinics, in many nations in the industrialized West has resulted in dramatic declines in incidence of most bacterial STDs in the last 10–14 years, particularly gonorrhea (1, 2). The situation in the United States is less optimistic. Whereas gonorrhea has declined by 40%, heterosexually transmitted syphilis, congenital syphilis, chancroid, and chlamydia have increased (3). Problems with these infections are even worse in many developing and underdeveloped nations in Africa, Asia, and elsewhere. Increasing antibiotic resistance in gonococci (3) and *H. ducreyi* (4) has necessitated use of newer, more expensive, antimicrobials, a circumstance that can prohibit effective therapy in developing nations.

The importance of the bacterial STDs rests in part on their adverse effects on reproductive health. Both gonorrhea and genital chlamydia are major causes of salpingitis, ectopic pregnancy, and infertility. A sense of urgency for the control of these diseases has emerged recently because of evidence that they significantly increase the rate of HIV transmission between sexual partners (5–9). Genital ulcer disease is recognized as an important cofactor for HIV transmission (7–9) and *H. ducreyi* and *T. pallidum* are prominent causes of the genital ulcer syndrome. The effects of gonorrhea and genital chlamydia on HIV transmission (5, 6) may be due to microulceration (10) and increased local accumulation of activated lymphocytes and macrophages, with a corresponding increase in release of HIV into genital secretions (11).

Because of the apparent roles of bacterial STDs in HIV transmission, the World Health Organization has concluded that strategies to control HIV should include development of effective programs for control of bacterial STDs (12). Although experience in several nations shows this can be accomplished (at least temporarily) without vaccines (1, 2), experience in other nations, including the United States, suggests that this is a difficult task. Vaccines against bacterial STDs would improve the public health and would offer long-lasting and cost-effective solutions to common and expensive problems. The question is, can such vaccines be developed? This paper briefly reviews relevant progress in research towards this goal.

CHANCROID

Chancroid is an ulcerating cutaneous infection caused by *H. ducreyi*. Infection may persist for months without effective antibiotic therapy. In earlier times, clinicians inoculated normal skin with pus taken from genital ulcers of the patient, and development of typical ulcerating lesions after such inoculation was used to confirm the diagnosis of chancroid (13). Indeed, Ducrey (13) was able to serially propagate the infectious agent at least 15 times in the same individual, indicating that little or no immunity occurs in naturally acquired infection. Today, diagnosis is made by *in vitro* culture. A renewed surge of research interest occurred after recognition that *H. ducreyi* is a cofactor for HIV transmission

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Abbreviations: HIV, human immunodeficiency virus; STDs, sexually transmitted diseases; mAb, monoclonal antibody; LOS, lipooligosaccharide; MOMP, major outer membrane protein; T_H, T-help.

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(7–9), and factors involved in pathogenesis are beginning to be delineated (14).

The cardinal feature of chancroid is the development of ulcers on stratified squamous epithelium, which suggests that *H. ducreyi* might produce cytotoxins or other tissue-destructive extracellular products. Recent evidence shows that many *H. ducreyi* isolates apparently do produce cytotoxins (15). Lagergard and Purven (16) found that injection of live *H. ducreyi* into rabbits resulted in low-titer anti-cytotoxin antibodies; a subsequent injection of live organisms resulted in neutralizing antibodies. Immunization of rabbits with crude preparations of cytotoxin (cell sonicates) resulted in neutralizing anti-cytotoxin antibodies that cross-reacted in similar titers with each of 12 heterologous cytotoxin-producing strains (16). Immunization with non-cytotoxin-producing *H. ducreyi* or other Gram-negative bacteria failed to produce cytotoxin-neutralizing antibodies (16). However, natural infection of humans apparently results in anti-cytotoxin antibodies, without evident protection from disease (16). The role of cytotoxin in the pathogenesis of human chancroid is unclear at present, and much remains to be learned about the genetics, biochemistry, and immunobiology of the putative cytotoxins. Interest in the cytotoxins is prompted, of course, by evidence that many other cytotoxins can be used to create effective vaccines, including diphtheria toxin.

A variety of other *H. ducreyi* proteins also have been identified, including outer membrane proteins and pili (14). Some of these proteins appear to be antigenically variable during infection of a subcutaneous chamber, a property that could explain the ability of *H. ducreyi* to persist *in vivo* (17). Little is known about the nature of the immune response in human chancroid, although there is evidence for both a T-cell (18) and a B-cell response.

SYPHILIS

The question of immunity in syphilis has long been the subject of investigation. Some level of protective immunity was suggested by early observations that only one-third of untreated cases of human syphilis developed late complications of disease (19). During early stages of active untreated infection, immunity to reinfection seemed to be present, the so-called "chancre immunity"; studies of experimental syphilis in rabbits confirmed this phenomenon (20). Development of chancre immunity is correlated with a vigorous humoral and cellular immune response to multiple antigens (21). After several weeks of untreated early syphilis, treponemes are cleared from the primary and secondary lesions. However, viable treponemes persist in untreated experimental animals for years in sites such as lymph nodes (22), suggesting that *T. pallidum* is capable of evading the immune response. Consistent with this is the protracted course of untreated disease in humans, with its characteristic waxing and waning in the first 1–2 years, followed by long intervals that ultimately (in one-third of cases) lead to late destructive lesions of the heart, central nervous system, or other organs.

Support for the possibility that an effective syphilis vaccine can be developed rests in large part on studies of experimental syphilis in human prison volunteers, conducted nearly 40 years ago at Sing Sing Prison in New York State by Magnuson *et al.* (23). They injected 10^5 (>100 times the ID_{50}) live *T. pallidum* harvested from rabbits into the skin of 62 adult males, 60 of whom were followed either until they developed lesions or until 4 months had elapsed. All were treated with penicillin at the end of the study. Each of 8 controls (no prior syphilis) developed dark-field-positive (spirochetes visible in dark-field microscopy) papules or ulcers at the inoculation site, whereas no lesions developed in each of 5 men with previously untreated latent syphilis. Among 11 men previously treated for early syphilis, 9 developed dark-field-positive lesions, and the other 2 developed dark-field-negative lesions. Among 31 men previously treated for late or congenital syphilis, only 2 developed dark-field-positive lesions, 15 developed dark-field-negative lesions (limited immunity?), and 14 appeared to be totally immune. Thus, durable immunity developed in many, but not all, men treated previously for natural infection, although at least several years of untreated infection were required to provide immunity.

Studies of immunity to *T. pallidum* in rabbits or hamsters confirmed the existence of slowly developed immunity. Both humoral and cellular arms of the immune system are important to immunity in animals (24–28). Antibodies with both neutralizing (27) and opsonic (28) activities, either of which could contribute to immunity, develop during infection.

Mechanisms by which treponemes persist in the presence of vigorous IgG, IgM, and cellular immune responses to multiple antigens (reviewed in ref. 29) are unknown. Studies of the physiology, biochemistry, and genetics of *T. pallidum* have been limited because the organisms still cannot be grown outside of animals, and because of lack of a genetic system for constructing mutants of *T. pallidum*. Antigenic structures seem stable during animal passage, and there is no evidence of the type of phase and antigenic variation or blocking antibodies that have confounded the search for a vaccine against gonorrhea. Persistence in the face of a vigorous antibody response could be due to intracellular localization, although the pathogenesis of *T. pallidum* infections seems to involve penetration between cells (30) rather than survival within epithelial cells or phagocytes.

One factor that may contribute to the persistence of *T. pallidum* is the apparent paucity of proteins in the outer membranes or envelope of the organism, which has been demonstrated by freeze-fracture techniques by two groups (31, 32). When compared with *Escherichia coli, T. pallidum* has only about 1% the density of integral outer membrane proteins. Proteins in the outer membrane of *T. pallidum* thus are termed TROMPs—treponemal rare outer membrane proteins. *T. pallidum* also lacks lipopolysaccharide in its outer membrane. Identities of TROMPs have been elusive to date, although searching recombinant *T. pallidum* libraries for *phoA* (alkaline phosphatase) fusions has identified *T. pallidum* genes that encode membrane-spanning proteins containing N-terminal signal sequences (33). Progress in the molecular characterization of *T. pallidum* genes and their predicted proteins by use of recombinant DNA technology is impressive (29).

Initial attempts to develop vaccines for syphilis employed the Nichols strain of *T. pallidum*, which fully retained its virulence for humans after decades of serial passage in rabbit testicles (23). Vaccination of rabbits with *T. pallidum* killed by either irradiation (34) or prolonged cold storage (35) resulted in immunity, but multiple large doses were required over considerable periods of time, limiting enthusiasm for analogous approaches in humans. Subsequently, Fitzgerald induced considerable immunity in rabbits with a single injection of heat-killed *T. pallidum*, when the animals were pretreated with cyclophosphamide and given various adjuvants (36). Fitzgerald concluded that whole-cell vaccines caused immunosuppression, which could be overcome by the sequential use of cyclophosphamide and adjuvants. No confirmatory results have yet been reported.

Current efforts to develop a syphilis vaccine focus on recombinant *T. pallidum* proteins. Three of these show limited efficacy in animals. An antigen designated TpN19 (or previously 4D) elicited partial protection in rabbits (37). TpN19 is interesting because in its native form it assembles into an oligomeric ringlike structure (38), although its function in pathogenesis or in the physiology of *T. pallidum* is

unknown. Vaccination with recombinant endoflagellar protein (39) or another protein designated either TpN36 or TmpB (40) also resulted in partial protection in animals.

These results do not permit conclusions about development of vaccines for syphilis, other than that some progress is being made. Difficulties in working with the organism are considerable, but application of modern biotechnology can be expected to hasten progress if funding for the few groups working on syphilis is stable.

GONORRHEA

Gonorrhea is a common disease and was known to ancient physicians. The quest for a vaccine is not new, and crude whole-cell vaccines were actually used as immunotherapy in the preantibiotic era (41). It is not possible to judge the efficacy of the old gonococcal vaccines because of insufficient description of the experiments and lack of controls, yet anecdotal experience suggested that modest doses of the homologous strain were useful (41). In more recent times, a variety of crude or partially purified antigens were used to vaccinate experimental animals against gonorrhea, with modestly encouraging results. For instance, immunization of chimpanzees with a whole-cell killed vaccine made from a chimpanzee virulent isolate conferred partial resistance to urethral challenge (42). Most current studies, however, are focused on purified and well-characterized antigens.

There are lessons to be derived from study of the course of untreated natural gonococcal infection. Symptomatic urogenital gonorrhea eventually subsided into a nonsymptomatic state in the preantibiotic era, and many patients became culture-negative and noninfectious for their partners (41, 43). By inference, the immune response was effective in controlling infection. However, immunity apparently was strain specific, since patients commonly reacquired gonorrhea many times. James Boswell's superb description of his 29 separate bouts of urogenital "gonorrhea" (some of which undoubtedly were due to *C. trachomatis*) illustrates the point (reviewed in ref. 44). Strain-specific immunity to genital gonorrhea was claimed in a recent study of recurrent gonorrhea in female prostitutes in Nairobi, most of whom were immunosuppressed because of infection by HIV. Reinfection was common, but was less likely to be due to strains exhibiting the same serovar of principal outer membrane protein, porin (Por) than by other Por serovars, suggesting partial immunity based on Por-specific epitopes (45). Subsequent work in the same population showed that presence of serum antibodies to Por was not correlated with protection (46). No attempt was made in the latter study to determine whether serovar-specific serum or genital Por antibodies to surface-exposed epitopes were correlated with protection from reinfection, which are the type of data one would desire, based on the earlier work.

Por as a Vaccine Candidate. Por is one of the antigens currently being studied as a possible vaccine. Por is the most abundant membrane protein, is expressed constitutively, and does not undergo high-frequency phase or antigenic variation *in vitro* or *in vivo*. Clinical isolates, however, do exhibit antigenic differences in some surface-exposed domains, presumably as a result of low-frequency mutations of *por* that allow escape from anti-Por antibodies (47). Some evidence suggests that Por participates in pathogenesis by translocating into eukaryotic cells (48), which might promote invasion, or in the case of neutrophils, apparently impairs neutrophil function (49).

Prior to the epidemiological studies of Nairobi prostitutes (45, 46), the possibility of a Por vaccine was suggested by Heckels and colleagues (50, 51), who showed that certain monoclonal antibodies (mAbs) against Por were bactericidal, stimulated chemiluminescence of neutrophils, and reduced toxicity to cultured epithelial cells. Moreover, some of the potentially protective mAbs reacted widely with either one or the other of the two main Por-based serogroups of gonococci—i.e., with strains of either the PorA or PorB serogroups (50, 51).

The *por* structural genes from several isolates have been cloned and sequenced (52, 53), allowing comparisons of the amino acid sequences of different Por molecules. PorA and PorB are closely related to each other and are products of variants of a single *por* gene (52, 53). There are multiple predicted membrane-spanning domains and models predict eight surface-exposed loops (54). By use of synthetic peptide technology, the common PorB epitope that reacts with the broadly protective PorB-specific mAb SM24 was defined as YSIP (55). The common PorA epitope that reacts with the broadly protective PorA-specific mAb SM101 (51) appears to be conformation dependent and cannot be localized to a single linear Por domain (53).

Elkins *et al.* (56) studied the immunobiology of polyclonal rabbit antisera raised against six synthetic Por peptides, four for PorA strain FA19 and two for PorB strain MS11. Results were encouraging in that polyclonal IgG purified from serum raised against the most N-terminal surface-exposed loop (loop 1) were bactericidal not only for the homologous strain but also for many other serological variants (serovars) of the same serogroup and to a limited extent for serovars of the other serogroup.

Purified Por also has been evaluated as a vaccine in animals, using *in vitro* bactericidal and opsonic assays as surrogates for possible protection of humans. Wetzler *et al.* (57) purified Por from gonococci containing a mutation in *rmp*, which encodes the reduction-modifiable protein (Rmp) formerly designated PIII; the importance of using an *rmp* mutant was in removal of the highly immunogenic Rmp antigen, which stimulates production of complement-fixing, but generally nonbactericidal, antibodies that block the bactericidal effects of anti-lipooligosaccharide (LOS) or anti-Por antibodies (58). Since mAbs against Rmp, Por, or LOS immunoprecipitate all three molecules, they apparently are tightly associated in clumps or patches in the outer membrane, helping to explain why antibodies against Rmp block bactericidal effects of anti-Por or anti-LOS. Using purified Por free of Rmp contamination, Wetzler *et al.* (59, 60) showed that Por liposomes were highly immunogenic and resulted in bactericidal and opsonic antibodies that recognized surface-exposed epitopes.

Elkins *et al.* (unpublished data) purified recombinant Por (rPor) expressed from an inducible promoter in *E. coli*. This approach has the potential advantages of permitting easy growth of large batches of cells, allowing use of novel hybrid PorA/PorB molecules (53), and also avoiding Rmp contamination. *E. coli* and other Gram-negative bacteria contain an outer membrane protein, OmpA, that exhibits significant C-terminal homology with Rmp (61), but rPor can be purified with <0.5% OmpA contamination, and anti-OmpA polyclonal sera do not react with Rmp (unpublished data). Immunization of rabbits with liposomal rPor purified with decyl β-D-maltoside so as to retain some conformational epitopes resulted in immune sera that bound well to Por on whole gonococci and exhibited opsonic, but not bactericidal, activity (unpublished data). Lack of bactericidal activity was not due to blocking antibodies, since gonococcal *rmp* mutants also were resistant to killing by anti-rPor rabbit sera.

A problem for all Por vaccines concerns the effects of sialylation of LOS. Sialylation of LOS, which lies physically proximate to Por in the outer membrane, abolished the bactericidal effect of anti-loop 1 peptide antisera by partially masking surface exposure of Por epitopes on whole bacteria and also by inhibition of complement activation (56). Sialylation of LOS also abrogated bactericidal and opsonophago-

cytic effects of anti-Por sera (60). Since gonococcal LOS is sialylated *in vivo* (62), vaccines based on these Por peptides (and possibly any Por antigens) might be ineffective. Blocking of certain bactericidal anti-Por mAbs by sialylation was incomplete, however, and could be overcome by use of more mAb (C.E., unpublished data). This suggests that killing might be possible with polyclonal anti-Por sera, if sufficiently high titers or high-affinity antibodies could be raised.

Recently, it has been possible to construct recombinant *Salmonella typhimurium* strains that express gonococcal Por constitutively from the *por* promoter without evident toxicity to the host strain (63). This should allow studies of the vaccine potential of Por delivered to the gut immune system and of the ability of Por to protect against homologous and heterologous infection in various (imperfect) mouse models of gonococcal infection (64). The advantage of this approach is that it should stimulate both humoral and mucosal immunity, both of which presumably are required for an effective gonococcal vaccine. It presumably will be possible to construct analogous strains in attenuated hosts suitable for use in humans, such as *Salmonella typhi*.

Ultimately, the utility of Por vaccines will have to be tested in humans. Recent studies of experimental urethral gonorrhea in male volunteers (65) show that such experiments can be conducted ethically and safely. Sialylation of LOS *in vivo* may effectively block any Por vaccine, but it should be possible to test this hypothesis directly in humans soon. If parenteral or oral vaccination with Por or peptides derived from Por results in protection against an ID_{80} intraurethral (male) challenge dose of the homologous strain, further consideration of Por vaccines will be warranted.

Pili as a Vaccine Candidate. Pili (Pil) are filamentous appendages composed of multiple pilin subunits, which function as adherence ligands. They appear to be necessary for infection, since Pil^- variants are essentially noninfectious in experimental infection of male volunteers, whereas Pil^+ gonococci are highly infectious under the same conditions (66). It was soon recognized that Pil undergo high-frequency phase and antigenic variation (67–70) and that Pil antigenic variants also exhibit different adherence properties (71, 72). The mechanism of many Pil variations involves recombination (69) between the *pilE* (structural gene) and one of multiple incomplete ("silent") variable copies of *pil* DNA present in several chromosomal loci (67–70). The rapidity and extent of Pil antigenic variation might help to explain how gonococci escape a vigorous immune response, and/or how gonococci rapidly adapt to adhere to quite different cells in diverse ecological niches (cervix, urethra). Sequencing of expressed *pil* genes from urethral isolates from previously uninfected males with experimental gonorrhea shows extensive variations, occurring so rapidly (first few days after inoculation) that immune pressure is an unlikely explanation (73) (H. S. Seifert, C. A. Wright, A. E. Jerse, M.S.C., and J. G. Cannon, unpublished data). Extensive antigenic variation obviously might make it difficult to produce an effective vaccine based on Pil, yet enthusiasm for a Pil-based vaccine once was high because of evidence that anti-Pil antibodies block adherence and are opsonic (72, 74, 75). Moreover, certain regions of the pilin subunit are relatively conserved (67, 75), and polyclonal rabbit sera against such partially conserved pilin peptides reportedly blocked *in vitro* adherence of heterologous gonococci (75). Enthusiasm lessened, however, when subsequent studies failed to confirm surface exposure of the conserved regions on intact pili expressed on whole gonococci (76). Moreover, extensive work in Heckel's laboratory showed that protective pilin mAbs all were directed at highly variable pilin epitopes (72, 74).

While these elegant studies of Pil variations and immunobiology were being conducted, several laboratories worked in a very directed fashion to implement a Pil vaccine for human use. Intramuscular injection of purified Pil resulted in serum (77) and genital IgG and IgA anti-Pil antibodies that blocked adherence (78) and, in a historical study, partially protected male volunteers from experimental urethral infection by the homologous strain (79). There was some evidence of development of cross-reactive anti-Pil antibodies (78, 79). A field trial of a vaccine composed of a single antigenic type of gonococcal Pil was conducted in Korea, and as might have been anticipated because of extensive Pil antigenic variation, there was no evidence of efficacy (80). Most investigators have concluded that Pil are subject to such a high degree of antigenic variation that a Pil-based vaccine is unlikely to succeed. It is conceivable that relatively conserved domains from a modest number of Pil variants might be useful in a vaccine, and limited research continues with the aim of exploring this possibility.

Other Possible Gonococcal Vaccines. Opacity proteins (Opa) are a family of up to 11 or 12 related outer membrane proteins that, like pili, also serve as adherence ligands, undergo high-frequency phase and antigenic variations, and appear to be necessary to establish genital infection (81). One or a few Opa proteins appear to promote invasion of eukaryotic cells (82). The molecular mechanisms for Opa variation are quite different from Pil variations. Opa expression is regulated by translational frameshifting, which is the consequence of high-frequency spontaneous variations in the number of a pentameric CTCTT oligonucleotide repeat present in the *opa* region encoding the signal sequence (83). Each of the approximately 11 *opa* genes (84) is constitutively transcribed, but most are not translated; at any time, a single cell expresses zero to four or rarely five Opas. Each Opa contains two regions of highly variable protein sequence (HV1 and HV2), and expression of different *opa* genes results in antigenic variation (83–85). Although mAbs against particular Opas decrease adherence (86, 87), no cross-reacting Opa antibodies have been described that either block adherence or have other potentially protective effects. Relatively little work has been done on possible Opa vaccines, influenced no doubt by considerations of difficulties encountered with the highly antigenically variable Pil vaccine. If subsequent work were to demonstrate that only one or at most a few Opas were required to initiate or maintain infection (which has yet to be proven), or if a common essential Opa domain were discovered, this situation could change.

LOS is another potentially useful immunogen. Gonococcal LOS has relatively typical core sugars, but no O antigen. The terminal core sugar is a close molecular mimic of certain host glycolipids (88). Antibodies against terminal core sugars are bactericidal, and many persons who have never been exposed to gonococci have serum IgM anti-LOS bactericidal antibodies against many gonococci (89). Since gonococci do not have a carbohydrate capsule (unlike closely related meningococci), LOS core sugars are the only carbohydrate antigens that might be considered for a vaccine. Unfortunately, evolution has enabled gonococci to escape attack on LOS, by at least three mechanisms. (*i*) Mimicry of host antigens reduces immune response to certain epitopes (88). (*ii*) Phase and antigenic variations affect the length and composition of core sugars and loss of terminal epitopes helps to evade antibodies against the core, although gonococci with a truncated core are complement sensitive. These variations occur at a frequency ($\approx 1 \times 10^{-3}$) similar to Pil and Opa variations (90). The genetics of LOS core variations are not well understood. (*iii*) Sialylation of the terminal core sugar occurs *in vitro* and *in vivo* (62, 91), when bacterial sialyltransferase and host-derived CMP-*N*-acetylneuraminic acid (CMP-NANA) as a substrate are used. Sialylation of the terminal LOS core sugar results in partial masking of both LOS and the physically proximate Por (56), inhibiting antibody attack on both LOS and Por. Sialylation also interferes

with effective formation of the terminal complement complex, thereby inhibiting bactericidal activity of antibodies against other nonmasked antigens such as Opa (56). Phase variation of core sugar composition results in transient loss of the terminal CMP-NANA acceptor site, and therefore, inability to undergo sialylation. This in turn seems to result in phase variation between a nonsialylated, invasive, but serum-susceptible, phenotype and a sialylated noninvasive serum-resistant phenotype (92).

On the basis of knowledge gained from studies of Por, Pil, Opa, and LOS, an ideal vaccine candidate might be one that does not undergo high-frequency antigenic variations, that is not protected by anti-Rmp blocking antibodies, and that is not masked by sialylation. It also should contain one or a few epitopes that are conserved and that are targets for protective antibodies. LOS seems a very unlikely candidate because of mimicry, phase and antigenic variation, protection by blocking anti-Rmp antibodies, and masking by sialylation. Pili seem to be too antigenically variable. Por remains a possible candidate because of relatively stable expression of cross-reactive epitopes for protective polyclonal and monoclonal antibodies and rather weak epidemiologic evidence for partial Por-based immunity, although partial protection by blocking anti-Rmp antibodies and LOS sialylation are major problems. Another candidate is one or both of the proteins that constitute the transferrin receptor. Transferrin-binding protein 1 (Tbp1) is a 100-kDa protein (93), and transferrin-binding protein 2 (Tbp2) is an unrelated 85-kDa protein. Together, these proteins constitute a specific and perhaps essential receptor for binding transferrin and removing iron from it (94). Expression of Tbp1 and Tbp2 is repressed by iron, and the structural genes *tbpA* and *tbpB* for Tbp1 and Tbp2, respectively, appear to be part of an iron-regulated polycistronic operon (J. Anderson, C. Cornelissen, and P.F.S., unpublished data). Comparison of the predicted protein sequences for Tbp1 in gonococci (93) and meningococci (95), and for Tbp2 in gonococci (C. Cornelissen, J. Anderson, and P.F.S., unpublished data) and meningococci (95) shows that these proteins are closely related. Antibodies against meningococcal Tbp1 and Tbp2 block meningococcal transferrin binding (96), cross-react rather broadly among meningococci (97), and are bactericidal[§]. Similar studies have yet to be conducted with gonococcal Tbp1 and Tbp2, but it is known that sialylation of LOS does not mask the gonococcal transferrin receptor (unpublished data of C. Cornelissen and P.F.S.). It is premature to do more than speculate about use of transferrin receptor proteins in a gonococcal vaccine, but the limited data are encouraging. IgA1 protease might also be considered on the basis of evidence that antibodies against meningococcal IgA protease cross-react broadly and block proteolytic activity (98).

CHLAMYDIA

C. trachomatis is an obligate intracellular bacterial pathogen that causes trachoma and genital tract infections. There are 15 principal serovars arranged into three serogroups. Serovars A, B, and C are responsible for most trachoma, and serovars D through K cause most genital infections (urethritis, cervicitis, salpingitis). Infection results in both protective immunity and tissue-damaging hypersensitivity responses, which has complicated efforts to develop vaccines. There has been remarkable progress in understanding the immunopathogenesis of chlamydia infections in the past decade, and a vaccine for genital chlamydia is now a realistic hope.

[§]Irwin, S., Jacobs, E., Danve, B., Quentin-Millet, M. J. & Schryvers, A. B., Eighth International Pathogenic Neisseria Conference, October 4–9, 1992, Cuernavaca, Mexico, p. 50 (abstr.).

Heat Shock Proteins and Hypersensitivity. Important lessons were learned from attempts to develop a vaccine against trachoma (reviewed in ref. 99 and 100). Ocular scarring and blindness result from chronic and repeated infection. Serovar-specific immunity develops over time, but other serovars remain infectious and trigger an apparent hypersensitivity response. Attempts to prevent infection with a crude whole-cell vaccine may actually have potentiated the hypersensitivity response after reexposure to infection. Protection of experimental animals from ocular infection was achieved with mAbs against the major outer membrane protein (MOMP), the serovar-specific typing antigen. An ocular hypersensitivity response was elicited in guinea pigs with the *Chlamydia psittaci* guinea-pig inclusion conjunctivitis agent (101), and topical application of one protein, a 57-kDa heat shock protein closely related to the *groEL* product (102), triggered a delayed hypersensitivity response in previously infected animals (103). Thus, the concept emerged that certain antigens such as MOMP may be useful in a vaccine, whereas others such as the 57-kDa GroEL homologue could be harmful in a vaccine.

Genital chlamydia infections also tend to be chronic and recurring and associated with scarring complications. Considerable evidence links chlamydia (as well as gonococci) to salpingitis, tubal infertility, and ectopic pregnancy. Tubal infertility may occur even in the absence of acutely symptomatic pelvic inflammatory disease, apparently due to smoldering and persistent chlamydia infection. Hypersensitivity to the 57-kDa GroEL homologue may contribute to complications of infections, since there is a strong correlation between presence of antibodies to this heat-shock protein and salpingitis (104), ectopic pregnancy (104), and tubal infertility (105). Indeed, antibodies to this protein may be predictive of women with chlamydia infection who will develop complications of infection (105). Women with salpingitis apparently also are more likely to have a delayed hypersensitivity response to the 57-kDa protein than other women with chlamydia infection (106). In infected primates, the pathology of diseased fallopian tubes is consistent with a cellular hypersensitivity response (107).

Both B-cell and T-cell responses probably are important to the protective immune response (99, 100, 108, 109). Interferon γ (IFN-γ) plays a prominent role in protection against genital chlamydia (110), and exciting recent evidence suggests that IFN-γ may result in a persistent, quiescent infection in which chlamydia express normal amounts of 57-kDa GroEL homologue but markedly decreased amounts of MOMP and other outer membrane antigens (111). If confirmed, this would help to explain how the immune response leads to chronic infection, and subsequently to a tissue-damaging hypersensitivity response to cross-reacting host heat shock protein antigens. Similar mechanisms may be involved in the arthritis–dermatitis–mucositis syndrome (Reiter syndrome) that often complicates genital chlamydia infection (112). It is unclear why the response is directed particularly to fallopian tubes, joints, and certain other tissues. Murine immune responses to chlamydial heat shock proteins are H-2 linked (113) which could help to explain why only a proportion of persons develop immune-related complications, assuming similar genetic control of anti-chlamydia responses in humans.

MOMP as a Protective Antigen. There is at least partial immunity to infection by the same strain after genital chlamydia infection in women (114), monkeys (115), and guinea pigs (116). Recovery of chlamydiae from the cervix is inversely correlated with presence of IgA antibodies reactive with *C. trachomatis* in cervical secretions (114), suggesting IgA may be protective for genital chlamydia. Antibody to several antigens is correlated with apparent protection against postabortal chlamydia salpingitis (117), but the best

evidence for antigen-specific protection concerns MOMP. Anti-MOMP polyclonal and monoclonal antibodies are neutralizing and protective against tissue culture infection by elementary bodies, the infectious extracellular form of chlamydia, and against toxic death in mice (118–121). Some MOMP mAbs neutralize more than a single serovar. Protection extends in some instances to subspecies specific epitopes but not to all serogroups of chlamydia.

These results led to the cloning and sequencing of the structural gene for MOMP from all serovars of *C. trachomatis* (122–124), with several important findings. MOMP is a transmembrane protein, with four surface-exposed and variable domains (VDI–VDIV) (125). Non-surface-exposed portions of the expressed protein are highly conserved. Proteolytic cleavage of VDII or VDIV reduces infectivity (126), as do mAbs specific for variable domain epitopes (127–129). By use of synthetic peptides, epitopes specific for neutralizing MOMP mAbs have been mapped to short linear peptides on the surface-exposed loops (100, 130). T-help (T_H) epitopes are located within conserved as well as variable domains of MOMP (131, 132), as well as on other chlamydia proteins (133).

A neutralizing epitope composed of seven amino acids located in MOMP VDIV, when coupled to a common T_H epitope composed of MOMP amino acids 106–130, elicited polyclonal antibodies in mice and cynomolgus monkeys that bound to the surface of *C. trachomatis* serovars D, E, F, and G, which cause about 80% of genital chlamydia infections, and neutralized tissue culture infectivity (129). Six of eight congenic mice that differed in major histocompatibility complex class II haplotype developed anti-VDIV antibodies after immunization, showing that there was not severe T-cell restriction in immune response to this peptide (129). These encouraging results suggest that a synthetic peptide vaccine against genital chlamydia is possible. An analogous peptide containing a T_H epitope and a VDI neutralizing epitope shows promise as a trachoma vaccine (134). The utility of purified recombinant MOMP as a vaccine also is being explored (135, 136).

MOMP does not appear to be protected by blocking antibodies or by strategies analogous to sialylation of LOS, both of which at least partially protect Por in gonococci. This suggests that it may prove to be easier to develop chlamydial MOMP vaccines than gonococcal Por vaccines. Both MOMP (124, 137) and Por appear to be subject to genetic drift, although how serious a problem this may be for vaccines is unclear.

Recently, a chlamydial 75-kDa outer membrane protein has been identified as a member of the heat shock protein 70 family (138) and more specifically as a DnaK homologue (139). Antibodies against this protein might be protective, since polyclonal antisera react with surface-exposed epitopes and are neutralizing *in vitro* (138). Moreover, there is a correlation between presence of antibodies to this antigen (as well as several others) and apparent protection from salpingitis (117). Unlike the 57-kDa GroEL homologue, the 75-kDa protein does not trigger an ocular hypersensitivity response in previously infected animals (140). Caution is warranted, however, because it shares regions of similarity with a sperm cell receptor (139).

PROSPECTS FOR THE FUTURE

It is much too early to predict future success for vaccines against any bacterial STD. Prospects appear best for a MOMP-based chlamydial vaccine, followed perhaps by a gonococcal vaccine based on Por and/or other antigens. It may be necessary to develop polyvalent vaccines, containing multiple epitopes of a single molecule such as MOMP or Por. This might be accomplished by synthetic peptide technology, or by use of recombinant DNA methods to construct novel chimeric proteins. It also may be necessary to combine different antigens, although this would greatly increase the number of possible vaccine candidate combinations to be tested. Lack of really good animal models limits development of vaccines for most of these infections, although there are several good models in which to study chlamydia vaccines. Delivery of a vaccine is another issue that will need to be addressed, and it could be crucial to initial efforts to determine whether a vaccine candidate holds real promise and is worth further development. Here, too, there is promise; a MOMP VDI neutralizing peptide for trachoma serovar A has been expressed in poliovirus, with evidence for strong immunogenicity in rabbits and production of high-titered neutralizing antibodies (141). The ideal vaccine would decrease transmission of the infection between partners and would prevent complications of disease.

Bacterial STDs are common infections and they cause important morbidity as well as potentiation of HIV transmission. The effort to develop vaccines to prevent these infections is challenging and is a very important goal.

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